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THE TRANSFORMATION OF PSEUDOGLOBULIN INTO EUGLOBULIN

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In several publications Banzhaf[1] states that when diphtheria serum is heated as it is in the preparation of antitoxin, part of the pseudoglobulin is transformed into euglobulin. Details regarding the methods of analysis, or the analytic data on which Banzhaf based his conclusion were not found in the publications examined.

This transformation has both a practical and a theoretical interest. It facilitates the concentration of the antitoxin present in the serum by removing protein without removing antitoxin, so that the final product contains all the antitoxin associated with much less protein. This is desirable because certain of the serum proteins have very little therapeutic value. In applying the heat treatment for the first time to a serum such as anthrax serum, for example, it is obviously desirable to be certain that a similar transformation takes place. Otherwise there would be no need of the heat treatment, and besides, the heating may cause loss of potency.

On the theoretical side, the fact that pseudoglobulin can be transformed into euglobulin without affecting the total number of antitoxic units is almost conclusive proof that the antitoxin is a substance separate from pseudoglobulin. That this transformation may take place in some sera, but not in all, is indicated by the following experiments, in which the heating of the serum was carried out under carefully controlled conditions and the analytic data obtained by improved methods. In all, four horse sera were used, which will be designated as follows:

Anthrax 48. The serum obtained from Horse 48; used in a previous work.[2] Potency high.

Anthrax 96. The serum obtained from Horse 96; used in a previous work. Potency lower than Serum 48.

Diphtheria 1. A mixture of sera obtained from 2 horses. Potency high.

Tetanus 1. A mixture of sera obtained from 2 horses. Potency fair.

The first attempts to detect the transformation failed almost certainly because the experiments were made in connection with the preparation of globulin for therapeutic use. Better analytic results were obtained when using smaller amounts of serum under conditions adapted to quantitative analysis and separate from antitoxin preparation.

Heating the serum.—The procedure with a single serum was as follows.

Into each of 2 erlenmeyer flasks of 200 cc. capacity, pipet 50 cc. portions of the serum. To each flask there were added 25 cc. of water and 32 cc. of saturated ammonium sulfate solution; resulting in 30% saturation. At this concentration euglobulin was precipitated. One flask, not to be heated, was stoppered and set aside. The other flask, to be heated in the water bath, was stoppered with a rubber stopper carrying a standard thermometer. The bath had been so adjusted that during the experimental heating of the serum mixtures, the bath temperature did not rise beyond 61°.2 nor fall below 60°.5. The temperatures inside the flasks (it was convenient to work with 2 sera at a time) rose from that of the room, about 27°, to 56° in the first five minutes of the heating, then to 59° in the next five minutes. After ten minutes heating the temperatures inside the flasks were exactly 60° or below it by a small fraction of a degree. The heating was then continued for exactly thirty minutes during which time the temperatures inside the flasks did not exceed 60°. At the end of the heating period the flasks were transferred to a pan containing cold water. This brought the temperature down to that of the room, in about five minutes. The next step is the separation and estimation of the precipitated euglobulin.

Method of analysis.—All four serum mixtures, 2 heated and 2 not heated, were transferred to 110 cc. centrifuge tubes and centrifuged for twenty-five minutes at about 2500 revolutions per minute. The sedimentation was perfect; the sticky euglobulin was firmly packed at the bottoms of the tubes. The supernatant liquids containing the pseudoglobulin, albumin and the antitoxin, were poured into 100 cc. volumetric flasks. The tubes could be inverted without loss of precipitate. The euglobulin was dissolved in water, coagulated by heat, filtered, dried and weighed by methods already described.[2] In the supernatant liquid estimations were made of (1) pseudoglobulin; (2) albumin and (3), these two together.

Results.—The figures in table 1 show unmistakably that more euglobulin precipitate was obtained from the heated than from the unheated serum. The figures for euglobulin in table 2, item (b), are the same as those in table 1, divided by 5. The main object of obtaining the other data was to ascertain the source of the excess of euglobulin in the heated precipitates and to ascertain how accurate or inaccurate the method of analysis was. Theoretically, in table 2, (a) should equal the sum of (b) plus (c), and (c) should equal the sum of (d) plus (e). Although this equality is absent, the differences appear to be consistent and indicate a uniformity of error due to the absence of corrections for the compara-

TABLE 1

WEIGHTS OF EUGLOBULIN OBTAINED FROM 50 CC. PORTIONS OF SERUM

SERUM	NO.	WITHOUT HEAT	WITH HEAT	INCREASE IN WEIGHT
		grams	*grams*	*grams*
Anthrax	48	0.774	1.201	0.427
Anthrax	96	0.547	0.793	0.246
Diphtheria	1	0.406	0.719	0.313
Diphtheria	1	0.435	0.642*	0.207
Tetanus	1	0.243	0.304	0.061

*Low result, due to incomplete flocculation.

TABLE 2

ANALYSES OF HEATED AND UNHEATED SERA. ALL WEIGHTS CALCULATED TO GRAMS IN 10 CC. SERUM

	ANTHRAX				TETANUS		DIPHTHERIA			
	48 not heated	48 heated	96 not heated	96 heated	1 not heated	1 heated	1 not heated	1 heated	1 not heated	1 heated
(a) Total coagulable protein	0.618*	0.618	0.788	0.788	0.558	0.558	0.665	0.665	0.665	0.665
(b) Euglobulin	0.155	0.240	0.109	0.159	0.049	0.061	0.081	0.144	0.087	0.128†
(c) Pseudoglobulin and albumin estimated together	0.437	0.378	0.668	0.625	0.447	0.451	0.534	0.487	0.523	0.484
(d) Pseudoglobulin	0.290	0.211	0.568	0.535	0.385	0.378	0.475	0.397	0.479	0.438
(e) Albumin	0.191	0.191	0.133	0.135	0.103	0.103	0.090	0.098	0.087	0.097
Pseudoglobulin converted into euglobulin	27%		6%		0.2%		16%		9%	

*The same figure for total coagulable protein applies to both the heated and not heated serum; these are not exact duplicates.

†Low result; see table 1.

tively large volumes of the precipitates, solubility of precipitate in the washwater, occluded ammonium sulphate, etc. All the errors however, do not invalidate a comparison of the data in one column with those on the same serum, in the next column.

The figures for albumin are practically the same in the heated and unheated sera. The figures for pseudoglobulin are consistently lower in the heated sera than in the unheated; this is an almost necessary consequence of the transformation of part of the pseudoglobulin into some protein having several of the precipitation characteristics of euglobulin. What is most important for the present investigation is that the *loss of pseudoglobulin in the heated sera corresponds almost quantitatively with the gain in euglobulin in the same sera.*

To calculate the percent of pseudoglobulin transformed into euglobulin by the heat treatment; the difference between the amount of pseudoglobulin in the unheated and heated serum divided by the amount of pseudoglobulin in the unheated serum gives the percent transformed; these are tabulated at the bottom of table 2. Thus for Anthrax 48, the figures are $(0.290-0.211)/0.290=27\%$. This is not the only way to calculate this figure. Figures for pseudoglobulin may be obtained from a different set of results; thus, they may be calculated by subtracting the figures for albumin (e) from those or pseudoglobulin plus albumin (c). If the percent of transformation be calculated from these lower values for pseudoglobulin the figures are, 24, 8.4., −1.2, 12.4 and 11.2%; to read across the bottom of table 2.

Further details are given in the *Journal of Agricultural Research*, 1917.

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[1] Banzhaf, E. J., *Proc. Soc. Exp. Biol. Med.*, **6**, 1908, (8). Also, in Collected Studies from the Bureau of Laboratories, Department of Health, City of New York, **7**, 1912–1913 (114), and **8**, 1914–1915, (209).

[2] Eichhorn, A., Berg, W. N., and Kelser, R. A., *J. Agric. Res.*, **8**, 1917, (37).

A CASE OF NORMAL EMBRYONIC ATRESIA OF THE ESOPHAGUS

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In a series of loggerhead turtle embryos, collected and used originally for a study of the history of the primordial germ cells, the esophagus was observed to be solid for a greater or less extent, approximately from the point of origin of the respiratory anlage to its bifurcation into the bronchi, from the twelfth to the thirty-second day of incubation. At the latter stage the esophagus is still occluded at its oral end, though now fenestrated for a considerable extent caudally; and it seems probable that the esophageal atresia persists practically to near the end of the incubation period (eight weeks) at the level just behind the opening of the larynx.

The points of special significance in regard to this material are: (1) the relatively longer persistence of the occlusion than has yet been described for any other form; (2) the absence of contributory yolk in the stenosed area; and (3) close relation of the atresia to the point of origin of the respiratory anlage, which fact may disclose its possible functional significance.

Balfour[1] was the first to describe a similar phenomenon in the esophagus of certain selachii. Kreuter[2, 3] confirmed these observations in the case of *Pristiurus* and *Torpedo*. Dean[4] reports a solid esophagus in larvae of *Amia calva*. An occluded esophagus is said to occur also in certain bony fishes, e.g., herring, trout, salmon (Balfour;